HATTREM

On intestinal putrefaction during water drinking with meals

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ON INTESTINAL PUTREFACTION DURING WATER DRINKING WITH MEALS

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WARNER MADISON HATTREM

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Warner Madison Hattrem

ENTITLED On Intestinal Putrefaction During Water Drinking with Meals

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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P. B. Hawk
per. W. A. N.

Instructor in Charge

APPROVED: W. A. Noyes

HEAD OF DEPARTMENT OF Chemistry

Introduction

It is well known that the intestine is the only internal organ in which, from the day after birth onwards, bacterial decomposition takes place continuously without the body suffering any necessary harm. The decomposition is of a chemical nature and consists in the fermentation of carbohydrates, the putrefaction of protein, and the conversion of fats into the lower fatty acids.

Under normal conditions, the fermentation of carbohydrates takes place in the lower part of the small intestine and in the upper part of the large intestine. Putrefaction, on the other hand, takes place only in the large intestine. The products of fermentation consist of gases, CO_2, H_2, CH_4, volatile fatty acids (acetic, butyric, etc.), and lactic acid. The fatty acids are either oxidized and expired, or eliminated unchanged in the urine. The fermentation products that are not absorbed are excreted with the feces or as flatus.

Putrefaction of protein produces ammonia, hydrogen sulphide and other gases, and also a number of other bodies such as aromatic oxy-acids, phenol, indol and skatol. The latter are absorbed by the intestinal epithelia and the gases are expired, while those substances which are absorbed are either excreted in the urine as compounds of sulphuric or glycuronic acid, or to a variable extent remain in the feces.

Baumann's investigations on the sulphur in urine were very extensive. He found and identified a number of different conjugated or ethoreal sulphates occurring in normal urine, and he also showed that they are all derived from aromatic products

formed in the intestines by the action of bacteria on the protein of the food. Accordingly, the ethereal sulphates in general and in indoxyl potassium sulphate particularly are supposed to indicate the degree of intestinal putrefaction.

According to Folin[1], in the sulphur, as in the nitrogen metabolism, there are certain regularities in the distribution of the waste-products. For example, he observed during an extended series of experiments on metabolism, that as the total urinary sulphur was reduced, the per cent represented by the inorganic sulphates decreased. This decrease was coincident with a decrease in urea for the same period. The reduction in the per cent of inorganic sulphur was accompanied by an increase in the percent of the total sulphur appearing as ethereal and neutral sulphur, the ethereal being more than doubled and the neutral increased four or five times. Considering total quantities, he found that the ethereal sulphates diminished as the total amount of sulphur diminished, but to a much smaller extent, and that the neutral sulphur was not visibly affected by the diminution of the total. In other words, the ethereal sulphate elimination was analogous to that of ammonia, uric acid or undetermined nitrogen, and the elimination of neutral sulphur resembled that of creatinine.

He interpreted these results on a basis of general processes of metabolism, and not on the basis of intestinal putrefaction. He found that under the influence of a nitrogen-rich diet, the indican and the ethereal sulphates did not vary to-

[1] Amer. Jour. of Physiology, 1905, 13 p. 98.

gether. Although the indican undoubtedly exists as an ethereal sulphate in urine, the experiments recorded by Folin speak against the view that the ethereal sulphates are all due to the same or similar conditions as give rise to the indoxyl sulphate.

Ellinger[1] showed that Blumenthal's views of indican being a product of tissue metabolism were incorrect, and today his proof is accepted by nearly everyone.

[1] Zeit. f. Phy. Chemie, 1903, XXXIX, p. 44.

From his analytical results therefore, Folin concludes that:-

1. The urinary indican is not, to any extent, a product of the general protein metabolism, and is therefore, probably, as is generally supposed, a product of intestinal putrefaction, and may be assumed to indicate approximately the degree of putrefaction in the intestinal tract.

2. The ethereal sulphates can only be in part due to intestinal putrefaction, and neither their absolute nor their relative amount can be accepted as an index of the extent to which the putrefaction is taking place in the intestines.

3. The ethereal sulphates, on the contrary, represent a form of sulphur-metabolism which becomes more prominent when the food contains little or no protein.

4. The neutral sulphur is not at all due to processes identical or similar to those which give rise to indican.

5. The neutral sulphur represents products which in the main are independent of the total amount of sulphur eliminated or of

the protein catabolized.

Now it is supposed that in ordinary intestinal digestion very little indol is produced; but wherever digestion is interferred with or delayed, as is the case in almost all intestinal diseases or in any condition preventing the passage of feces through the small intestine, the formation of indol is greatly increased. Indol on further oxidation in the body forms indoxyl or some indoxyl compound, and this oxidation is associated with, or followed by, a synthesis with sulphuric acid, occurring mainly in the liver and partly in the muscles, and which results in the production of indoxyl potassium sulphate which is then eliminated in the urine. Not all of the indol goes to form indican. Some is excreted with the feces, hence the amount of indican in the urine does not represent the total amount of indol formed, but if data is collected over a long period of time, the indican content of the urine will give a rough indication of the putrefaction taking place in the intestinal tract. The presence of indican in the urine in large quantities is of great clinical significance, but since its daily output may be influenced by such a variety of conditions, much caution should be observed in drawing conclusions from its presence.

It was the purpose of the experiments which follow, to investigate the influence of water drinking with meals upon the course of intestinal putrefaction. The output of indican in the urine was taken as the index of such putrefaction. The total ethereal sulphate output was also determined in one experiment for purposes of comparison.

Experimental. Influence of copious water drinking. For this purpose a normal male subject, W, was used. He was fed a uniform diet until nitrogen equilibrium was obtained. The menu for each of the three meals of the day was as follows:

Graham crackers	150 grams
Peanut butter	20 grams
Butter	25 grams
Milk	450 c.c.
Water	100 c.c.

Three additional volumes of water of 200 c.c. each were taken at 10 A. M., 3 P. M., and 8:30 P. M., respectively. The experiment covered a period of ten days, consisting of a preliminary period of two days, a water period of five days and a final period of three days. During the water period an additional 1000 c.c. of water were taken at each of the three meals of the day.

The urine was collected in twenty-four hour samples. The feces were also passed regularly once a day. This latter feature is of prime importance in experiments in which determinations of urinary indican are to be made, inasmuch as retention of feces would naturally be followed by the absorption of added quantities of indol and a consequent increased output of indican. The urine was examined for indican according to the quantitative method of Ellinger[1], and for ethereal sulphates according to the

[1] Zeit. f. Phy. Chemie, 1903 - 38 - p. 192.

method of Folin.

Ellinger's Method for Indican. - There is at present no method for the quantitative determination of indican which can be said to be absolutely reliable. Ellinger's method as used in these experiments is accepted today as the most accurate, but even it has its sources of error. The method employed is as follows:-

Fifty c.c. of urine are placed in a small beaker and if neutral or alkaline in reaction should be made faintly acid with acetic acid. Five c.c. of basic lead acetate[1] is then added to precipitate the phosphates and coloring matter, the solution is mixed well and filtered. Transfer 40 c.c. of the clear filtrate to a separatory funnel, add an equal volume of Obermeyer's reagent (2-3 grams ferric chlorid per liter conc. HCl) and extract the indigo thus formed with chloroform.

This extraction with chloroform should be repeated until the chloroform solution remains colorless. Filter the chloroform extract through a dry filter paper into a dry Erlenmeyer flask. Distil off the chloroform, heat the residue on a boiling water bath for 5 minutes in the open flask, and wash the dried residue with hot water until the water is no longer colored. Add 10 c.c. of concentrated sulphuric acid to the washed residue, heat on the water-bath for 5-10 minutes, dilute with 100 c.c. of water and titrate the blue solution with a very dilute solution of potassium permanganate. The end point is indicated by the dissipation of all the blue color from the solution and the formation of a pale

[1] U. S. Pharmacopoeia.

yellow color.

Ellinger claims that one-sixth of the amount determined must be added to the value obtained by titration in order to secure accurate data.

[1] Practical Physiological Chemistry. - Hawk.

In making up the permanganate for titration an approximate 0.3 per cent solution was first prepared. This stock solution was then diluted with forty volumes of water, as suggested by Wang[2]. The dilute solution was then standardized and employed in the titration. One cc = 0.176 mg indigo.

[2] Wang: Zeit. f. Phy. Chemie, 1898 - 25 - p. 409.

In starting these experiments, 50 c.c. of urine was used. The chloroform extract appeared thick and seemed full of small globules. These were so persistent that the solution would not filter. Some of these small globules were examined under the microscope and were found to contain beautiful blue crystals. They were identified as indigo crystals.[3] The solution when evaporated without filtering, left a heavy brownish residue in the flask which could not be washed out without loss of indigo. This residue was due to small amounts of the supernatent liquid being entrapped between the globules of the chloroform extract and drawn off with it. This difficulty was finally overcome by using only 25c.c. of urine and diluting it to 50 c.c. When using urine from

[3] Imabuchi: Zeit. f. Phy. Chemie, 1909, 60, p. 502.

moderate water-drinking experiments, 50 c.c. could be used without any difficulty. On the other hand urines from the copious water periods were concentrated in order to insure the presence of enough indigo to make a satisfactory determination.

Another difficulty was in getting a good blue solution in the chloroform. It was noticed in the first experiments that the chloroform solution was a bluish red color. The residue after distilling off the chloroform was reddish brown, and on dissolving in sulphuric acid and diluting with water, gave a red solution which could not be titrated with potassium permanganate with any degree of accuracy. Upon investigation it was found that the source of the trouble was the thymol which was used as a urine preservative.

Making the determinations on fresh urine before the preservative was used, resulted in a clear blue chloroform solution which upon evaporation left the indigo in fine concentric circles on the bottom of the flask. It was very easy now to wash this residue without the danger of loss of indigo. Failure to wash the residue properly left some of the red coloring matter, probably isatin[1], which gave the solution which was to be titrated,

[1] Ellinger: Zeit. f. Phy. Chemie, 1903, 38, p.188

either a greenish or turbid blue appearance. This somewhat hindered the determination of the delicate end point.

Methods for the determination of indican have been reported by various investigators including Bouma, Folin and Imabuchi.[2]

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[2] Bouma: Zeit. f. Phy. Chemie, 1901, 32, p. - 82.

Folin: Amer. Jour. of Phy., 1905, 13, p. 53.

Imabuchi: Loc. Cit.

Bouma boiled the urine with hydrochloric acid containing isatin, thereby changing all the indoxyl of the urine into indigo red. Much of the other detail was the same as Ellinger's method. He finally titrated a red solution against permanganate, which certainly is anything but satisfactory as regards the securing of a visible end point. He reports the formation of crystals of indigo red in the examination of urines of high indican content.

Folin used practically all of Ellinger's method except the final dissolving in sulphuric acid, dilution and titrating. He extracted the indigo with 5 cc. of chloroform and then determined the amount colorometrically by a comparison with Fehling's solution which he gave an arbitrary value of "100". The comparisons were made by means of a Duboscqu Colorimeter. In our experience this proceedure does not yield as satisfactory results as the method of Ellinger. The procedure of Folin has not yet been placed upon a strict quantitative basis.

Imabuchi conducted his work in much the same manner as did Ellinger, except that he used a few cc. of a 10 per cent solution of copper sulphate with 40 cc. of concentrated hydrochloric acid to oxidize the indican to indigo blue, whereas Ellinger used Obermeyer's Reagent. By an exhaustive series of experiments, he showed that with copper sulphate it was possible to obtain more indigo, and that it was not necessary to extract

immediately with chloroform as was the case when Obermeyer's reagent was used. His results varied only slightly after the solution had stood for a period of ten minutes, whereas longer standing caused lower results. He also determined that the best amount of copper sulphate solution to use was between 1 and 3 cc., 0.5 cc. being insufficient to oxidize all of the indican to indigo and 5 cc. causing superoxidation. He claims that excess of reagent is less harmful in the case of copper sulphate than with Obermeyer's reagent.

A few parallel determinations were made in connection with our experiments in which the efficiency of Obermeyer's reagent was compared with that of copper sulphate. The results obtained by us were practically the same in each instance.

Discussion.

It may be seen from Table I, p. 24, that the amount of indican in the urine was nearly the same for each of the two days preceeding the copious water drinking with meals. On the first day of the five day water period, there was a decided drop in the output of indican, the lower daily excretion continuing with but slight variation for three days. The last two days of the period show, on the other hand, marked increases which, however, did not continue into the final period. The results for the final period agree fairly well with those of the first three days of the copious water period, showing that the effect of the copious water must have been carried over into this period.

The effect is better observed by noting the average

values for each period. The avera e for the copious water period, 33.4 mg., is lower than that of the preliminary period, 39.1 mg., thus indicating that copious water drinking with meals decreases intestinal putrefaction. The average for the final period was lower than that of the copious water period, showing that the effects of the increased water ingestion persisted even after the water quota had been reduced to the preliminary level.

This point is shown very nicely by an examination of Table I, p. 24 . After the completion of the experiment on copious water drinking, Subject W, upon his return to the usual mixed diet, drank rather larger quantities of water at meal time than had been his custom previous to the time he served as a subject in our tests. Three months later, wishing to learn the exact reaction of his organism as regarded intestinal putrefaction when he was ingesting a diet of the same character as that employed in our water study, this individual was placed on such a diet and the urine examined. The data show that the daily average value for this seven-day period was 37.1 mg. as against 39.1 mg. for the period under the same conditions three months previous. There was, therefore, slightly less intestinal putrefaction. It is of interest to note also that the value as obtained was considerably higher than the daily average for the final period of the copious water drinking study, i.e. 27.0 mg. This might be interpreted as indicating that the markedly inhibited putrefaction processes had been augmented by degrees above this final value during the period since the copious water ingestion, but that the conditions surrounding these processes had been so

altered through this excessive intake of water as to require the lapse of a period of three months before they were enabled to assume their former activity.

Now, if, as Baumann claims the total ethereal sulphate excretion is an index of intestinal putrefaction, we should obtain a decreased output of this form of sulphur during the period of copious water drinking.

Table I, p. 24, shows the total daily output of ethereal sulphates for the interval during which indican determinations were made. The average value for the preliminary period was found to be 0.1341 gram. On the first day of the copious water period there was an increase in the output to 0.1500 gram, while the average daily value for the entire period was considerably higher than that of the preliminary period, i.e. 0.1460 gram. The first day of the final period showed an increase from 0.1479 gram to 0.1580 gram, while the general average 0.1538 gram for the period was higher than that of the copious water period.

A comparison of the ethereal sulphate data with the indican values for the same urine fails to reveal any uniformity in the rate of excretion in the two instances.

The indican values <u>decrease</u> during the water period and are still further depressed in the post-water interval, two observations which have been interpreted as indicating a progressively decreasing putrefaction. On the other hand, the output of total ethereal sulphates is <u>increased</u> during the water period and still further increased during the period following the water.

In other words, the course of the ethereal sulphate

excretion is directly opposite to that of the indican. It is logical to conclude therefore, that they cannot correctly be considered as indices of the intensity of the same metabolic process. This point is all the more significant when we consider that indican is itself an ethereal sulphate and therefore, forms a part of the total output of this form of sulphur, hence the increase in the oth r forms of ethereal sulphates, except indican, was greater than the data show, inasmuch as the increase occurred during an interval in which the indican was considerably lessened in amount. These results lead us to believe that Baumann's statement, that the ethereal sulphates may be taken as an index of intestinal putrefaction, is incorrect.

Influence of Moderate Water Drinking. - Following the investigation of the course of intestinal putrefaction as influenced by copious water drinking with meals, a second experiment was undertaken, in which the topic of moderate water drinking was under consideration.

The added volume of water daily ingested with meals was only one-half that ingested per day during the study of copious water drinking, i.e., 500 c.c. This volume was deemed representative of so-called moderate water drinking. Two subjects were employed one of whom, W, had already served as a subject in the copious water drinking experiment. The other subject, E, was accustomed to drinking rather large amounts of water with meals. In fact, throughout the course of the investigation he frequently said he was ingesting less water than he ordinarily ingested when living on an ordinary mixed diet. The experiment on this sub-

ject, E, may, therefore, be considered as embracing the study of the influence of moderate water drinking with meals upon a confirmed water drinker. The daily program of the experiment was similar to that of the experiment on copious water drinking.

Subject W ingested the following diet three times daily:--

Graham crackers	125 grams
Butter	25 grams
Peanut Butter	20 grams
Milk	400 c.c.
Water	100 c.c.

An additional volume of 200 c.c. of water was taken at 10 A. M., 3 P. M. and 8 P. M., respectively. His experiment was divided into a preliminary period of three days, a ten day period of moderate water drinking, and a final period of twelve days during which the same conditions were in force as during the preliminary period.

Subject E used a similar diet to that already mentioned in connection with W, with the exception that his menu contained twenty-five grams of graham crackers additional per meal. He also drank an additional 200 c.c. of water at 10 A. M., 3 P. M. and 8 P. M. The experiment covered the same number of days as the experiment on W, but the periods were divided somewhat differently. It consisted of a preliminary period of eight days, a moderate water period of ten days, and a final period of four days.

Discussion of Moderate Water Drinking. - It may be seen

from Tables II and III that there is a decrease in the average daily output of indican from the preliminary periods to the moderate water periods of both subjects. Also in both cases there is a noticeable increase as soon as the original low water ingestion is resumed. In the case of W for instance, the preliminary output was 45.3 mg. per day, a value which was lowered to 42.5 mg. under the influence of the moderate water drinking. When the low water ingestion was again instituted, upon the first day of the final period, there was an increase in the indican output for three days, followed by three days in which the output was below the level even of the water period. Taking the entire final period into consideration, we obtain a daily average of 47.9 mg. as compared with one of 45.3 mg. for the preliminary period. However, if we calculate upon the basis of the last four days of the final period, we secure an average of 43.0 mg. which is lower than the preliminary level and practically the same as the daily output for the water period.

The data from subject W indicate that moderate water drinking exerted an inhibitory influence upon intestinal putrefaction. However, upon the withdrawal of the added water from the diet, the inhibitory factor being thus removed, there was no check to intestinal putrefaction and this process which had been held in inhibition by the water, again had full sway. This was noted during the first part of the final period. As the period progressed, however, conditions surrounding the putrefaction reactions became altered to such an extent as to yield an average daily value of practically the same magnitude as that secured during the water period.

From a comparison of Tables I, p.24 and II, p.25, it will be seen that on the same subject, W, the effect of moderate amounts of water with meals was not as pronounced as with the copious water drinking. In the copious water experiment, there was a gradual decrease in the total daily output of indican which did not increase during the final period. In the moderate water experiment the amount of indican excreted daily decreased slightly during the water period. There was then an increased output during the first part of the final period which was not shown in the copious water experiment. These facts seem to indicate that there was less intestinal putrefaction during the copious ingestion of water with meals than when using only a moderate amount of water. Although there was a noticeable decrease during the moderate water period, the average daily output varied only slightly from day to day, showing that the moderate amount of water did not have the pronounced effect which was in evidence during the experiment on the copious water ingestion.

Subject E, Table III, p.25, showed a more pronounced effect with moderate water ingestion than did subject W. From an average daily excretion of 69.3 mg., the value for the first day of the water period fell to 51.4 mg., with a general average of 47.9 mg. for the entire period. The output for the first day of the final period rose to 57.5 mg. with a general average of 60.5 mg. for the entire period.

Now Subject E, it will be remembered, was the subject accustomed to ordinarily ingesting larger amounts of water than he was using during the preliminary period of this experiment.

In the face of this fact it would seem that the additional 500 c.c. of water taken during the moderate water period acted in a manner similar to that in which the copious amounts of water acted on Subject W.

Or we may suppose the lower water ingestion during the preliminary period of this subject to have increased the intestinal putrefaction, and that later during the moderate water drinking the normal level for this individual was reached. This may be the true explanation inasmuch as the subject frequently remarked, that the added 500 c.c. ingested with each meal during the water period, was no more than he was accustomed to take. In the final period the increase during the four days may be interpreted as was the high value for the preliminary period, i.e., as due to the fact that the subject wasnot receiving as much water as he needed to keep his putrefaction processes properly regulated. That the value for these days is not as high as the preliminary level is probably due to the after effects of the inhibitory influence of moderate water drinking.

The average daily output of indican for Subject E is seen to be greater than that of Subject W throughout the experiments. This may be due to the factor of individuality. For instance, it has been pointed out by Herter[1] that the number of indol producing bacteria in the intestinal tract varies with different subjects.

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[1] Bacterial Infections of the Digestive Tract, p. 263.

Influence of Copious Water Drinking on a Confirmed Water Drinker.

Subject E, as has already been mentioned, was accustomed to drinking fairly large volumes of water at meal time. For this reason it seemed that the data secured from copious water ingestion by him might yield interesting findings, particularly when taken into comparison with similar data obtained from Subject W.

At the close of the final period of the moderate water experiment he was, therefore, given a five day preliminary period on the same diet. This was followed by a five day copious water period, during which he ingested an additional 4000 c.c. of water per day. Then followed a three day final period with the regular low water ingestion. An inspection of Table IV, p. 24, will show the results of this study.

The average amount of indican excreted daily in the urine during the preliminary period, was 67.3 mg. During the first two days of the copious water ingestion, there was a large decrease in the daily excretion with an exceedingly large output in the third day. The remainder of the period decreased again with an average for the period of 54.1 mg., showing a rather pronounced inhibition of intestinal putrefaction during the ingestion of large amounts of water. The output of indican during the final period increased with an average daily excretion of 68.2 mg., a value slightly higher than the preliminary value.

Again it is shown that the ingestion of large amounts of water with meals decreases the putrefaction in the intestinal

tract. Here as in the case of moderate water drinking the putrefactive processes were much more active in the case of Subject E than in the case of Subject W. In fact this variation in the reaction of the two organisms in this respect was not limited to the water period, but was markedly in evidence throughout the various portions of the investigation. An interesting fact is brought out when the percentage decreases in the output of indican during copious water drinking are calculated for subjects E and W. Notwithstanding the actual output per day was greater in the case of E, the percentage decrease brought about in the indican excretion through the ingestion of the large volume of water was greater in his case. The values are 19.6 per cent decrease for Subject E against a decrease of 14.6 per cent for Subject W. It will be remembered that the diet was practically the same for the two subjects, the only variable factor being the water, of which W ingested 3000 c.c. additional per day during the water period, whereas E's added intake was 4000 c.c. The latter subject also ingested 150 c.c. less of milk per day than did W. It may be that this extra liter of water ingested by E over that taken by W, may have been the efficient factor in causing a more marked lessening of intestinal putrefaction in the case of that individual. We would not expect a priori, however, that the ingested water would prove as efficient in this regard in E's organism or in that of W inasmuch as the former was an habitual water drinker.

The pronounced increase in the output of indican upon the third day of copious water drinking by subject E has already

been mentioned. When this value was first obtained it was deemed incorrect, and the urine was then again analyzed. Repeated analyses yielded similar values. An explanation was finally found when the feces data were examined. These data showed that the average daily output for this subject had been about 135 grams of moist feces per day for a long period previous to this time. On the first day of water drinking the output was only 90.3 grams, whereas on the second day the output was only 37.2 grams. There was, therefore, evidently a retention of feces in the intestine, for on the next day, the third of water drinking, a stool weighing 249.4 grams was dropped. On the dry basis, conditions were similar to the above, as the data indicate that the average normal output of dry feces per day had been about 32 grams for some time.

Under the influence of water this value was decreased progressively to 18.2 grams and 10.2 grams upon the first two days of water drinking, values which were followed by an output of 58.8 grams of dry matter upon the third day. Inasmuch as the urine sample tabulated as the urine of the third day of water drinking was the urine collected between 7:15 A. M. on the third day and 7;15 A. M. on the fourth day, and further, inasmuch as the third stool of the water period which we have mentioned as weighing 249.4 grams and having a dry matter content of 58.8 grams was also passed on the morning of the fourth day of water drinking, it is evident that this large mass of feces was in the intestine during the interval in which the urine of the high indican content was being passed into the bladder.

The undue retention of this fecal mass gave more oppor-

tunity for the indol forming bacteria to complete their task, thus causing the ultimate passage of an extra quota of indican into the urine.

A case very similar to this occurred during the moderate water drinking experiment on Subject E (see Table III, p.25) On the fifth day of the preliminary period there was an extremely low output of indican, far lower in fact, than for several days preceeding and following. An examination of the feces data shows that on the fourth day of the period a stool weighing 193.9 grams was passed, whereas the stool for the fifth day weighed only 76.9 grams and was followed on the sixth day by a stool weighing 207.7 grams. The dry weights of the stools for these days were 47.8, 20.1, and 47.3 grams, respectively. Thus from the same reasoning as that advanced above it is seen that the lower output of indican on this fifth day must have been due to the fact, that on the fourth day of the period the gut was more completely evacuated than usual, as shown by the defecation of a stool weighing 193.9 grams as against an average of 135 grams for the period. This unusually large output of feces therefore, left but a comparatively small fecal residue within the intestine. The indol forming micro-organisms consequently were not able to produce the customary daily quota of indol and, therefore, the low value for urinary indican was noted.

The last stool passed during the moderate water drinking period of Subject W and the stool passed on the third day of the copious water drinking period of Subject E, were both acid in reaction to litmus. W's stool weighed 152.7 grams against an aver-

age output of 105.3 grams. The stool was thus about 50 per cent larger than the average daily output. Notwithstanding the fact, however, the indican output for the corresponding urine was only 34.7 mgs., the lowest daily output for the period. The acid reaction of the intestinal contents during the time the B. Coli and other indol formers were producing the indol output, evidently lowered the efficiency of the micro-organisms in this respect. Hence less indol was available for subsequent detoxication, conjugation and final elimination as the indoxyl potassium sulphate.

In the case of the acid stool in E's water period, the conditions were somewhat similar. This stool was passed about 15 hours sooner than it should have been in the usual course of events. On the morning of the day in question, a stool weighing 74.7 grams was passed and in the afternoon a stool weighing 258 grams and possessing an acid reaction was dropped. There were therefore, 332.7 grams of feces passed during the day as against a daily average of 152.5 grams, yet the urine corresponding to this fecal output showed an indican value of but 48.0 mg. which was nearly 50 per cent lower than that of the previous day. Two factors evidently were instrumental in bringing about this result. In the first place, the stool was defecated 15 hours sooner than usual, thus leaving less of an interval for the micro-organisms to accomplish the putrefaction of the protein residues; and, in the second place, the acid reaction of this stool would tend to lessen the efficiency of these bacteria even during the shortened time at their disposal.

These two factors easily account for the markedly lessened indican output.

Conclusions.

1. The drinking of copious (1000 c.c.) or moderate (500 c.c.) volumes of water with meals decreased intestinal putrefaction as measured by the urinary indican output.

2. Copious water drinking caused a more pronounced lessening of the putrefactive processes than did the moderate water drinking.

3. In copious water drinking the total ethereal sulphate output was <u>increased</u> coincidently with the <u>decrease</u> in the indican output. This observation furnishes strong evidence in favor of the view that indican has an origin different from that of the other ethereal sulphates, and that they cannot correctly be considered as indices of the same metabolic process.

4. When Ellinger's method is employed, the determination of indican should be made on fresh urine before any preservative has been introduced. Especially is this true when thymol is used as the preservative.

Table I

Subject W

No.	Urine Volume	Indican (Mg.)	Grams Ethereal SO_3
	Preliminary Period		
1	888	38.0	.1409
2	945	40.1	.1273
Av.		39.1	.1341
	Copious Water Period		
3	3110	26.9	.1500
4	4570	25.3	.1618
5	4230	28.5	.1257
6	3810	40.9	.1446
7	3300	45.5	.1497
Av		33.4	.1460
	Final Period		
8	1100	33.6	.1580
9	930	22.6	.1489
10	965	24.8	.1545
Av.		27.0	.1538
	Three Months Later		
1	578	36.9	
2	710	31.7	
3	825	30.2	
4	715	43.6	
5	828	40.7	
6	785	44.6	
7	772	32.1	
Av.		37.1	

Table IV.

Subject E.

No.	Urine Volume	Indican (Mg.)
	Preliminary Period	
1	992	72.3
2	940	71.8
3	920	71.9
4	858	63.3
5	980	57.4
Av.		67.3
	Copious Water Period	
6	4550	30.9
7	4785	53.1
8	5220	86.0
9	4530	48.0
10	4850	52.6
Av.		54.1
	Final Period	
11	970	60.6
12	1009	68.3
13	880	75.8
Av.		68.2

Table II

Subject W, Moderate Water

No.	Urine Volume	Indican (Mg.)
Preliminary Period		
1	740	54.2
2	807	41.4
3	777	40.3
Av.		45.3
Moderate Water		
4	2086	40.2
5	2412	44.4
6	2415	46.0
7	2560	44.5
8	1485	46.9
9	1660	39.5
10	1800	42.6
11	2336	41.5
12	2242	44.8
13	1720	34.7
Av.		42.5
Final Period		
14	1370	68.8
15	760	46.5
16	781	47.6
17	700	42.4
18	783	40.8
19	650	40.6
20	760	55.8
21	793	60.9
22	690	45.6
23	757	35.1
24	677	42.1
25	757	49.2
Av.		47.9

Table III

Subject E, Moderate Water

No.	Urine Volume	Indican (Mg.)
Preliminary Period		
1	1065	42.1
2	937	62.0
3	1047	77.9
4	1031	75.0
5	1062	38.6
6	1143	94.5
7	1301	105.3
8	1250	58.2
Av.		69.2
Moderate Water		
9	2192	51.4
10	2465	45.5
11	2395	57.6
12	2110	60.6
13	2220	26.9
14	2760	37.7
15	1745	50.8
16	2277	46.6
17	2078	49.9
18	1090	52.1
Av.		47.9
Final Period		
19	870	57.5
20	885	53.8
21	945	61.1
22	998	69.8
Av.		60.5